# Preclinical evaluation of the recombinant dendritic cell growth factor CDX-301 (Flt3L), and AST-008, a TLR9 agonist SNA

#3217

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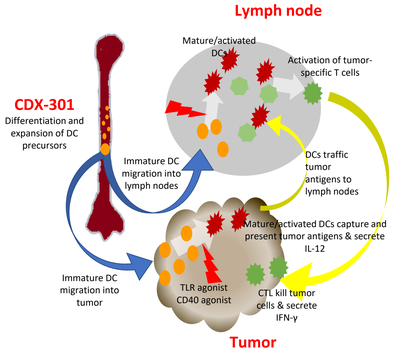
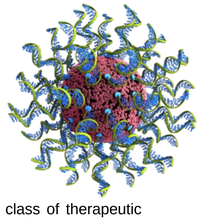
## BACKGROUND: CDX-301

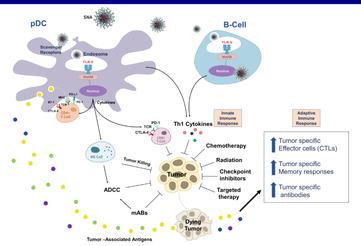
- Dendritic cells (DCs) are often rare or completely missing from tumors and are necessary for anti-tumor immunity.
- CDX-301 is the soluble recombinant human protein form of the Fms-related tyrosine kinase 3 ligand (Flt3L), a hematopoietic cytokine.
- Flt3 receptor (CD135) is expressed on hematopoietic stem cells, early progenitor cells, immature thymocytes, and steady state dendritic cells.
- CDX-301 has shown an ability to increase the number of DC precursors and DCs in blood and tissue, including the CD141$^+$(BDCA3$^+$) cDC subset in humans and the corresponding CD103$^+$ cDC1 subset in mice.
- In humans and mice, the intratumoral CD141$^+$/CD103$^+$ DCs are important for antigen cross-presentation to T cells and correlate with improved outcomes for multiple tumor types.




## BACKGROUND: AST-008

- Toll-like receptor 9 (TLR9) belongs to the family of pattern recognition receptors in the innate immune system and is predominately expressed in B cells and plasmacytoid dendritic cells (pDCs).
- CpG dinucleotides present in specific nucleic acid sequence contexts induce immune responses via stimulation of TLR9.
- Spherical Nucleic Acids (SNAs) are a novel class of therapeutic agents with oligonucleotides densely packed and radially oriented around a spherical liposomal nanoparticle.
- As a result of the 3D architecture, SNAs exhibit increased cellular uptake and primarily accumulate in endosomes where TLR9 is expressed.
- AST-008 and muAST-008 are human- and mouse-selective TLR9-agonist SNAs





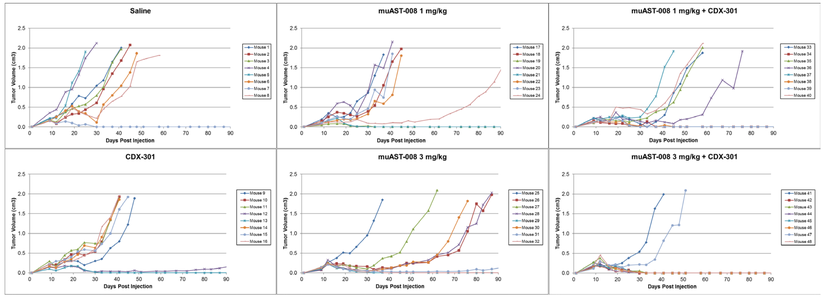

TLR9 agonist SNAs induce Th1-type cytokine production, plasmacytoid dendritic cell (pDC) maturation, and pDC and B cell activation. Subsequent activation of T cells and NK cells results in tumor killing and release of tumor-associated antigens leading to tumor-specific adaptive immunity. In addition, other therapies can be combined with SNAs to enhance the cancer therapy.

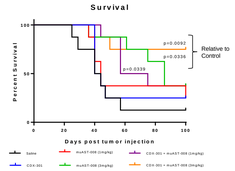
## CONCLUSIONS

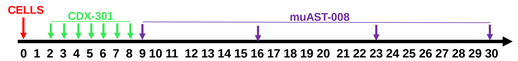
- Treatment with CDX-301 and muAST-008 showed an additive effect in retarding tumor growth and prolonging survival.
- We observed a significant increase in the percentage of splenic CD103$^+$ cDCs from the addition of muAST-008 to CDX-301 treatment.
- In addition, muAST-008 led to the up-regulation of activation markers on dendritic cells, which was markedly enhanced when combined with an agonist CD40 antibody.
- These data demonstrate that muAST-008 leads to systemic activation of CDX-301 expanded DCs, leading to more potent anti-tumor immunity and support the potential of combining CDX-301 and AST-008 in augmenting the immunotherapy of cancers.

## EFFICACY IN MC38 MODEL

**A**



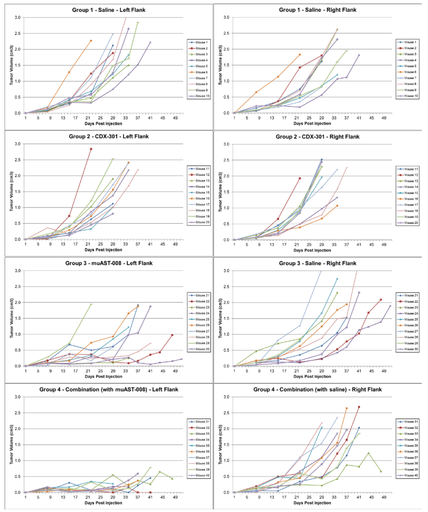




**A.** Female C57BL/6 mice received $10^6$ MC38 cells s.c. in one flank on Day 0. Animal were divided into Group 1, receiving saline on days 9, 16, 23 and 30 p.t.; Group 2, receiving 5µg CDX-301 on days 2-8 i.p.; Group 3, receiving 1mg/kg muAST-008 on days 9, 16, 23 and 30 p.t.; Group 4, receiving 3mg/kg muAST-008 on days 9, 16, 23 and 30 p.t.; Group 5, receiving 5µg CDX-301 on days 2-8 i.p. and 1mg/kg muAST-008 on days 9, 16, 23 and 30 p.t.; and Group 6, receiving 5µg CDX-301 on days 2-8 i.p. and 3mg/kg muAST-008 on days 9, 16, 23 and 30 p.t.

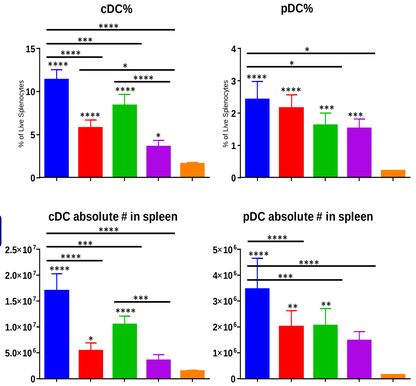
**B.** Female C57BL/6 mice received $10^6$ MC38 cells s.c. in both flanks on Day 0. Animal were divided into Group 1, receiving saline on days 9, 16, 23 and 30 p.t.; Group 2, receiving 5µg CDX-301 on days 2-8 i.p.; Group 3, receiving 3mg/kg muAST-008 on days 9, 16, 23 and 30 p.t. in the left flank only; and Group 4, receiving 5µg CDX-301 on days 2-8 i.p. and 3mg/kg muAST-008 on days 9, 16, 23 and 30 p.t. in the left flank only.

**B**

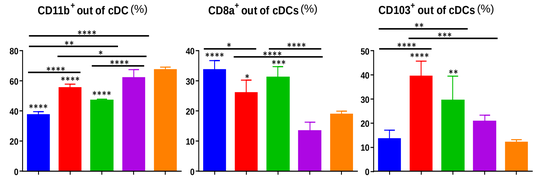



## DC PROLIFERATION AND ACTIVATION

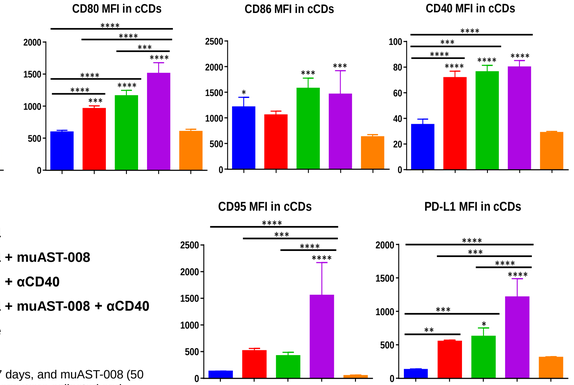
### DC EXPANSION

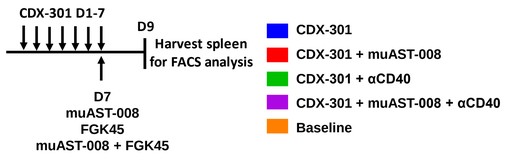



### cDC SUBTYPES




### DC ACTIVATION




DC proliferation and activation. C57BL/6 mice were treated with CDX-301 (2.5 ug, i.p.) daily for 7 days, and muAST-008 (50 ug, s.c.) or agonist anti-CD40 antibody FGK45 (50 ug, i.p.) on day 7, as indicated. On day 9 spleens were collected and processed into a single cell suspension, and stained for DC subset and activation markers. DC subsets were defined as follows; cDC = CD11c$^{++}$MHC-II$^{++}$, pDC = CD11c$^+$MHC-II$^+$B220$^+$.